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Selective Inhibition of the Synthesis of Sindbis Virion Proteins by an Inhibitor of Chymotrypsin

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Treatment of chick embryo fibroblasts infected with Sindbis virus with TPCK, the choloromethyl ketone derivative of tosyl-phenylalanine and an inhibitor of chymotrypsin, resulted in reduced synthesis of viral structural proteins and the accumulation of a high-molecular-weight polypeptide, thought to be a precursor. The analogous inhibitor of trypsin, TLCK, the chloromethyl ketone derivative of tosyl-lysine, had no such effect.

Work in several laboratories has clearly established that the virus-determined proteins of various enteroviruses are produced by the cleavage of high-molecular-weight polypeptides that represent the primary gene products [reviewed by Baltimore (1)]. The nature of virus-specific protein synthesis in cells infected by arboviruses is less clearly understood. The amino acid analogues that allow a clear demonstration of the poliovirus precursor proteins (5) do not significantly affect the electrophoretic pattern of virus-specific proteins in cells infected by Sindbis virus (6). Similarly, very short pulses of labeled amino acids gave little hint of a precursor protein in Sindbis infections (6) although both positive (2) and negative (3) results have been reported for Semliki Forest virus.

Prompted by the use of diisopropylfluorophosphate, a nonspecific inhibitor of proteolytic enzymes, to reveal a large precursor of poliovirus proteins (4), we turned to more specific antagonists of chymotrypsin and trypsin. TPCK and TLCK, the chloromethyl ketone derivatives of tosyl-phenylalanine and tosyl-lysine, are known to be potent noncompetitive inhibitors of chymotrypsin and trypsin, respectively (7, 8). Although these antagonists significantly inhibit protein synthesis by Sindbis virus-infected chick embryo cells, they can be used to explore the process of virus-specific protein synthesis. On the basis of preliminary experiments, we chose concentrations of TPCK (20 μg/ml) and TLCK (50 μg/ml) that produced about the same inhibition of ^{3}H-leucine incorporation. At these concentrations, neither substance was virucidal.

Figure 1 shows the effects of TLCK and TPCK, each added 4.5 hr after infection, on the rates of protein synthesis and virus release. Both inhibited

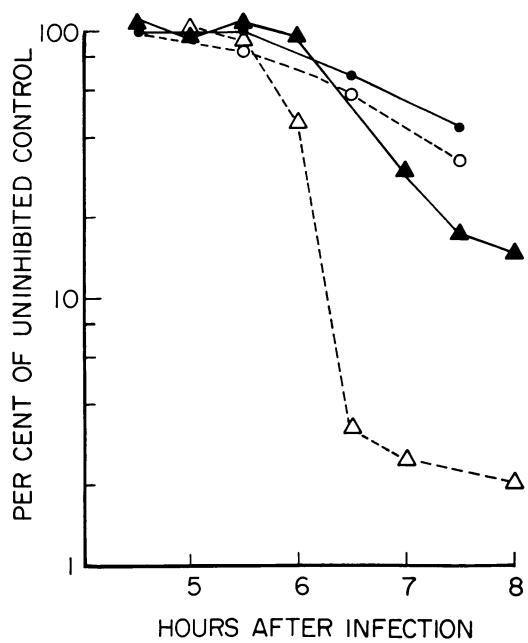

FIG. 1. *Effect of TPCK and TLCK on the incorporation of ^{3}H-leucine and the release of virus. Replicate chick embryo fibroblast cultures were infected with Sindbis virus at 50 plaque-forming units (PFU)/cell and incubated in medium containing 10 μg of L-leucine/ml. After 4.5 hr, some cultures were exposed to medium that contained TPCK (20 μg/ml) or TLCK (50 μg/ml). The rate of protein synthesis was measured by incubating the cultures in fresh medium containing 3 μCi of ^{3}H-leucine/ml and determining the acid-precipitable radioactivity of triplicate cultures 50 min later. The rate of virus production was determined from the PFU titers of medium that was changed every 30 min. Symbols: TLCK-treated, ^{3}H-leucine incorporation (●); TLCK, virus release (▲); TPCK, ^{3}H-leucine incorporation (○); TPCK, virus release (△).*

the incorporation of ^{3}H-leucine to about the same extent. However, TPCK, the inhibitor of chymotrypsin, proved to have a much more dramatic effect on the production of virus, an effect so great that it could not be explained simply by the gross inhibition of amino acid incorporation.

Examination of the synthesis of virus-specific proteins in cells inhibited by TPCK revealed a possible explanation of the marked reduction in virus yield. Acrylamide gel electrophoresis (Fig. 2) showed that TPCK-treated cells accumulated a slowly migrating high-molecular-weight protein and produced diminished quantities of both viral structural proteins. In contrast to the action of TPCK, the analogous inhibitor of trypsin, TLCK, had no effect on the electrophoretic pattern of virus-specific protein (Fig. 3).

Interpretation of these data is obviously complicated by the inhibition of amino acid incorporation by both inhibitors of proteolysis. Note that the absolute amount of slowly migrating high-molecular-weight polypeptide is the same in control cells and in those inhibited by TPCK (Fig. 2). Thus, the possibility remains that TPCK, by some unknown mechanism, specifically inhibits the synthesis of low-molecular-weight proteins. We believe that a more likely explanation, however, is that the inhibition of ^{3}H-leucine

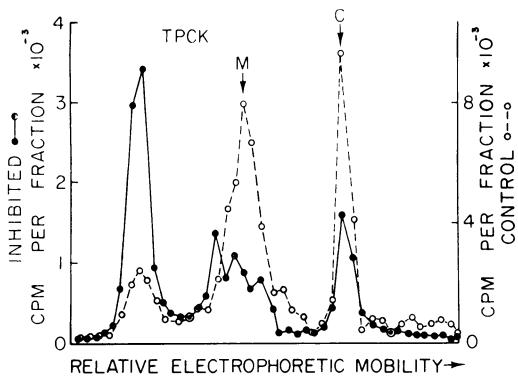

FIG. 2. *Effect of TPCK on the synthesis of virus-specific proteins. Replicate cultures were infected with 50 plaque-forming units of Sindbis virus per cell and incubated in medium containing 1 μg of L-leucine/ml. At 4.5 hr, one culture was supplemented with TPCK (20 μg/ml). Between 5 and 6 hr after infection, both the inhibited and control cultures were labeled with fresh medium containing 30 μCi of ^{3}H-L-leucine/ml. The cells were then prepared for electrophoresis on sodium dodecyl sulfate-acrylamide gels as previously described (6). A small amount of ^{14}C-amino acid-labeled, purified Sindbis virus was added to each extract before electrophoresis to supply markers for the viral membrane protein (M) and the viral capsid protein (C). The electropherograms of the inhibited and control cultures have been superimposed by alignment of the ^{14}C-marker peaks (data not shown).*

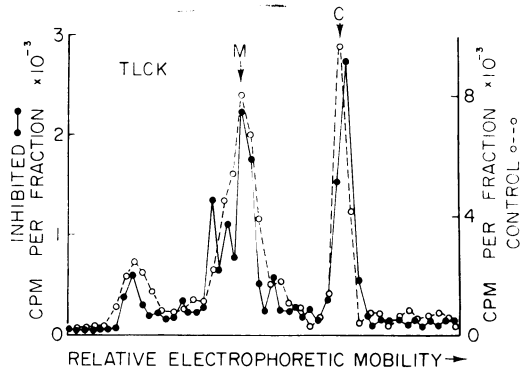

FIG. 3. *Effect of TLCK on the synthesis of virus-specific proteins. The experimental procedure was the same as that of Fig. 2 and the same control served for both. TLCK was used at a concentration of 50 μg/ml.*

incorporation is a nonspecific toxic effect of both TPCK and TLCK. Superimposed upon this effect, we then see the accumulation of a precursor protein and reduced production of viral structural proteins when proteolysis is inhibited by TPCK. The small amount of protein in control cells that has about the same electrophoretic mobility as this supposed precursor may represent incomplete cleavage or may be an unrelated protein of similar molecular weight. If our reasoning as to the mechanism of TPCK action is correct, at least some of the cleavage of Sindbis virus precursor proteins is carried out by an enzyme having the specificity of chymotrypsin.

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